analysis

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EDITORIAL

THE INTEGRITY OF SCIENCE

Throughout the centuries, science has functioned within a framework of immutable laws. The objectivity of these laws has lodged within the public consciousness a willingness to accept scientific findings at face value. Consequently, nothing is so perplexing to the public as the spectacle of eminent scientists disputing the interpretation of scientific data.

The confusion arises from the fact that the scientist has failed to clarify for the public the various roles he is required to play. No longer does the scientist work independently; he or she is generally employed by industries, institutions, or governments to pursue specific tasks. The employer is often in a position to exert significant pressure on the form and content of research findings.

The scientist must be prepared to rigidly separate scientific fact from speculation, opinion, and personal or employer bias. Adroit or unwitting manipulation of these ingredients, resulting in pseudo-scientific conclusions, damages science and misleads the public. Scientists can avoid becoming latter-day sophists or paid sycophants by scrupulously avoiding these pitfalls.

A less obvious but perhaps equally insidious danger confronts the scientist whose data interpretation is subjected to administrative review prior to publication. The review process can be transformed into a "laundering exercise" wherein every finding deemed controversial is emasculated or edited out.

The scientist has a duty and responsibility to ensure that in the interests of a "balanced view", the significance of any particular scientific study does not become obscured. Every scientist has inherited a hard-won trust upon which the integrity of science is sustained. Scientists, individually and collectively, must jealously guard and nurture this trust. It is no longer enough to possess a trained scientific mind to serve science. Vigilance coupled with mental and moral toughness are essential for scientists to function adequately in today's society.

DISASTER CHEMISTRY

Just before midnight, on Saturday, November 10th, 1979, Canadian Pacific Rail's Train 54 derailed at the Mavis Road level crossing in Mississauga, Ontario. The cargo, consisting mainly of propane, but also including liquid chlorine, styrene, toluene, butane and caustic soda, was engulfed in flames. Despite the efforts of the Mississauga firefighters and the flood of water they directed at the flames, the ensuing explosions and the risk of exposure to chlorine resulted in the most extensive mass evacuation in North American history.

Although the chemical ingredients became known during the early phase of the accident, the estimation and prediction of reaction products was made very difficult by the uncontrolled nature of reaction sequences and conditions. The enormity of the problem can be grasped by considering that there were tank car quantities of liquid chlorine and chlorine gas, butane, propane, styrene, toluene, and 50% aqueous sodium hydroxide, in close proximity to flame, heat, light and plenty of water.

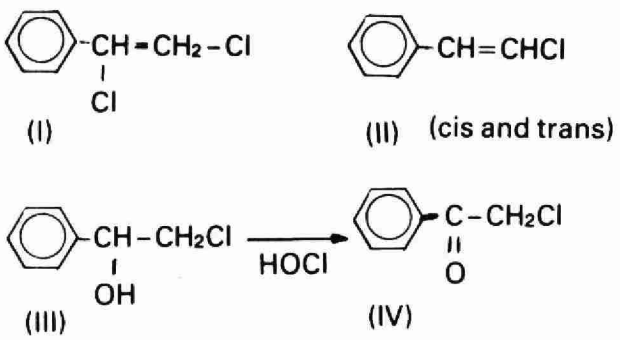
Within hours of the occurrence of the explosion, the Ministry deployed two mobile monitoring units to the site. The Air Resources Branch Taga 3000 and the Sciex Taga II began monitoring the ambient air to track the dispersion of the escaped gases. These units maintained around the clock monitoring of the air at the site and in the surrounding neighbourhoods for a period of nine days.

After the flames were extinguished, soil and water sample collection at the site became possible. The accident site was approached with caution as it was suspected that about twenty tons of liquid chlorine (with a boiling point of $-34^{0}C$) were still in the tank, encrusted with a layer of crystalline chlorine hydrate several inches thick. Water, mud and soil samples were collected and analyzed in the laboratory. Gas purging and solvent extraction, followed by gas chromatography /mass spectrometry (GC/MS) were used to separate and identify organic contaminants.

Considering the nearly limitless possibilities of chemical transformations under the circumstances, the contamination picture unveiled by analysis was surprisingly simple. No traces of chlorinated propanes, butanes or toluenes were found. All chloro-organics identified were derived

from styrene and chlorine. Besides unaltered toluene and styrene, the major organic component was styrene dichloride (1,2-dichloro-phenylethane) (I) followed by w-chlorostyrene(II). The other, lesser constituents, could be divided into two groups: chlorinated compounds derived from styrene dimer and those from styrene trimer.

Altogether, some twenty components were found, and all but one contained chlorine. The unchlorinated component was identified as phenylacetaldehyde, most likely a secondary, hydrolysis product of w-chlorostyrene. Styrene chlorohydrin (III) was found in one of the earliest samples but not afterwards. As the pH of the water and soil in the area ranged from 12 to 13, this compound may have undergone further hydrolysis. Since a fairly strong lachrymal atmosphere lingered around the accident site for days, the presence of the crowd control chemical "Mace" (w-chloroacetophenone) (IV) was suspected, and the Taga unit verified its presence by MS in the air samples. While the formation of this compound from styrene, chlorine and water is quite feasible, none was found by GC/MS in the soil and water samples which were analyzed later. It is possible that the air also contained the dichloro compound, a known lachrymating agent (I).



It is interesting to note that none of the compounds found contained chlorine attached to the benzene ring.

Extensive analytical work was directed to follow some runoff headed for the Credit River and to monitor clean-up operations.

Ministry staff theorized that the bulk of the chlorine gas, approximately 70 tons, escaped under pressure when the chlorine tank burst. It was also speculated that the combination of pressure and the chimney effect resulting from the force of the explosion were factors in shifting the chlorine away from ground level and facilitated its transportation and diffusion up to 20 miles from the explosion site.

It appears from the results of the chemical analyses that a significant portion of the small amount of chlorine that escaped at ground level, was "neutralized" by the styrene. One ton of styrene has the capacity to react with 2/3 of a ton of chlorine.

The wind direction that prevailed at the site throughout the alert period after the explosion was also a key ingredient in mitigating the potential harmful effects. The combination of all these fortuitous factors was largely responsible for the fact that the citizens of Mississauga suffered no discernible environmental health effects from the disaster. The capable manner in which the disaster and subsequent evacuation of the area were handled by the authorities and the citizens was a credit to all concerned.

(O. Meresz/R. Bonner (416) 248-3031)

TOMBSTONES UNDER ATTACK

United States researchers are investigating the effects of acid precipitation on stone monuments and statuary. A novel feature of the study relates to the use of marble tombstones as indicators of past environmental damage.

The researchers required uniform material located in a variety of climates and environments over a continuous period of time. Marble tombstones appear to be an ideal target material.

Since 1875, the U.S. Veterans Administration has provided over 2.5 million tombstones to various National Cemeteries. These tombstones have been relatively standardized, and are made from stone taken from only three quarries. These standard conditions provide researchers an excellent opportunity to document the effects of acid precipitation on stone.

The researchers hope to be able to evaluate such effects as measurable loss of detail, rounding of edges, and surface erosion to develop quantitative estimates of damage. These data will then be correlated with data on the stone's history from Veterans Administration records and data on air pollution and meteorological patterns from the National Weather Service.

POLLUTION INDICATORS IN FECES OF BEAVER

Studies of the fecal flora of beaver (Castor canadensis) are not numerous and therefore very little information is available on the makeup of enteric organisms in these animals.

Wildlife can act as a reservoir of bacterial disease infections such as Tularemia, Pseudotuberculosis, and Listeriosis, which are all highly infectious and dangerous to man. These diseases have also been recorded in beavers and beavers have been implicated in waterborne outbreaks of Giardiasis. Bacterial transmission to man can occur in several ways, the most important being through contaminated drinking or bathing water.

The question of significance of beaver and their bacterial intestinal flora has arisen with the recent growth of beaver populations, particularly in areas being used for recreational purposes.

This study was undertaken to determine the pollution indicator bacteria that are associated with beaver feces. Not only would identifying fecal matter indicate the possible presence of bacterial pathogens, but protozoan parasites like Giardia, which themselves are difficult to enumerate, could also be indicated.

In this study, fecal samples were taken from two beavers trapped by a local licensed trapper in Buck Lake. 32 samples of feces and intestinal lining were aseptically removed and enriched in either Tryptic Soy Broth (TSB) or Brain Heart Infusion Broth (BHI). In addition, Skim Milk Broth tubes were inoculated with samples from the second beaver. The enriched samples were incubated at 35°C for 24 hrs. after which they were plated on to a variety of media. Membrane filtration was carried out on fecal samples from the second beaver. A quantity of 1 gm of feces was suspended in 100 mL of buffered diluent and a series of dilutions of this suspension were placed on m-Endo Agar, m-Enterococcus and m-MacConkey Broth plates. The identification of coliforms was carried out using the API identification system.

The API identification revealed a number of fairly common gastro-intestinal organisms. There was a distinct lack of Klebsiella species which was surprising since Klebsiella are often linked with wood and pulp processing. Mannitol Salt Agar showed a number of Staphylococcus which were associated with the intestinal lining more frequently than the feces. The organisms were equally spread between both the large and small intestine. Membrane filtration, carried out on the second beaver, failed to recover any total or fecal coliforms. The fecal streptococci isolated were unlike those found in most water samples; they were smaller colonies and were of light pink colour.

The total recovery rate of coliforms on MacConkey broth in the second sample was only 16%. The physical appearance of the feces in the second sample was markedly different, being much looser and wet, suggesting that the animal may not have been in the best of health and represented an atypical sample. A further study by the MF procedure of the beaver feces would be required to be able to assess whether the poor recovery was due to poor sample or to the inability of the MF method to recover indicators from such samples.

(P. Racey (613) 549-4000)

THE DETERMINATION OF LEAD IN DRINKING WATERS BY HYDRIDE GENERATION, IN THE PRESENCE OF COPPER AND NICKEL

The method for the analysis of lead in environmental samples by hydride generation and flameless atomic absorption spectrometry (HY-FAAS) has been in use for several years. In the method, gaseous hydride is generated by a reaction between tetravalent lead in a 0.7% nitric acid solution (v/v) and a 4% solution of sodium borohydride (w/v). The hydride is swept into a heated quartz cell, after passing the reaction mixture through a gas-liquid separator and drying the gases over concentrated sulphuric acid. The quartz cell is pre-aligned in the light path of an atomic absorption spectrometer equipped with a lead hollow cathode lamp, and set at a wavelength of 217 nm. The method is automated by means of a Technicon sampler, proportioning pump and manifold. Concentrations of 3 µg/L Cu and 10 µg/L Ni respectively are known to completely obliterate the lead signal when these elements are simultaneously present in the sample.

Recently, the need arose to determine lead in potable waters from a nickel mining

and smelting district. These waters contained elevated levels of copper and nickel, so the conventional HY-FAAS method had to be modified to analyze these samples. In the modified method, lead is co-precipitated along with manganese dioxide, formed by the addition of manganous sulphate and potassium permanganate to the acidified sample. The precipitated manganese dioxide quantitatively removes lead from the solution leaving copper and nickel in the supernatant liquid, which is discarded. The precipitate is dissolved in 0.85% nitric acid solution (V/V) and analyzed as before. The proposed method was compared to conventional flame atomic absorption spectrometric (AAS) and differential pulse anodic stripping voltametric (DPASV) methods in order to corroborate the data; acceptable agreement was found at all levels tested.

The accuracy of the method was determined by the replicate analysis of the United States Environmental Protection Agency's standard reference samples. Results are shown in the table.

(P. Vijan, R. Sadana (416) 248-3446)

Lead in Standard Reference Material (μg/L)

Sample No.	Certified Value	AAS	HY-FAAS	DPASV
EPA 1	22	24	22	21
EPA 2	300	320	307	330
EPA 3	350	360	356	320

REDEFINING THE TERMINOLOGY OF "ERROR" FOR SCIENTISTS

The term "error" has been assigned conflicting interpretation by various users. To the layman, an error is a mistake; to the statistician, an error is only a deviant value (relative to a central value) caused by random effects which are said to follow the "normal law of error" (i.e. not a mistake). Statistical methods place a great deal of emphasis upon the identification of outliers (i.e. points not following the "normal law of error") which are supposed to be deleted from the data set before applying further statistical tests. Often it is assumed that these "outliers" are not "mistaken" results but rather that they simply represent another data set which has a different central value. The analytical scientist is well aware, however, that mistakes occur. Therefore we require language which will differentiate between outliers which can be validated and those which cannot. Several of the following terms are often considered to be synonymous but there is considerable advantage in retaining the flavour suggested by the attached definitions.

Deviation: acceptable variation in the results of measurement processes as defined by experience and/or the "normal law of deviations"

Standard Deviation: when the data set follows the "normal law of deviations", the root mean sum of squared deviations

i.e. $s = \sqrt{(\Sigma d^2)/(n-1)} =$ standard deviation

where $d = x - \bar{x}$

and $\bar{x} = (\Sigma x)/n$ = mean

can be used to describe the distribution of deviations from the mean.

Repeatability: is an indication of the range of deviation resulting from within-run random variation in procedure for a single analyst.

Reproducibility: indicates the deviation resulting from both within-run and between-run activities for a single analyst.

Precision: indicates the deviation resulting from within-run, between-run, and between-analysts activities.

Error: unacceptable variation based on criteria such as arithmetic mean and multiples of the standard deviation. In order to demonstrate that some results differ greatly from others will require replicate analyses and/or knowledge of the "correct" value.

Indeterminate Errors: are those which cannot be reproduced on reanalysis (e.g. spattering during sample digestion) and which can be prevented only by care and training.

Determinate Errors: are those which can be reproduced (e.g. interference effects) and therefore detected and corrected, but which are related primarily to the handling and preparation of the sample, and which therefore affect different samples individually.

Systematic Errors: are those resulting from improper calibration and/or use of measurement instruments, which are then applied to all samples analyzed within the same "run". This error may vary significantly from one "run" to another unless controlled. If it is detected, a correction factor for the "run" can be determined and applied. There is no need to reanalyze all the samples.

If both systematic and determinate error are controlled, reproducibility and precision should not exceed about 1.5 times the repeatability. By reversing this statement one could define compatible analysts as those whose combined precision is not significantly larger than their average individual repeatability. (Successful control over reproducibility within a laboratory would be indicated by a similar ratio against repeatability). If on the basis of interlaboratory comparison the reported precision for a sample is significantly larger than that expected from single analyst repeatability one can deduce that non-compatible data (either less repeatable or biased) has been included.

Bias: Systematic error in one or both of two sets of data will result in a detectable bias between the two data sets on average. The bias may occur between-run, between-method, or between-analyst. Comparison of sets of paired data will often indicate that bias can be described in terms of a linear equation covering the range of data.

In many instances instrument calibration requires the estimation of a zero point and a line (curve) which describes the sensitivity (result per unit response) over the operating range. A systematic error in the assignment of zero is often accompanied by a compensating error in the sensitivity (slope). There will then be a narrow range of measurement within which the total error vanishes against the background deviation. The absence of detectable bias is not proof of lack of systematic errors.

Inaccuracy: If one of two data sets is known or can be defined to be "true", then any bias between them is a measure of inaccuracy.

It is inappropriate to make accuracy statements on the basis of the analysis of an external reference material when there is no accompanying statement indicating control over both within-run repeatability and between-run reproducibility. It is also not appropriate to assess accuracy based upon data at only one or two points over the range.

There are two ways in which external reference materials can be used by the analyst to assess accuracy; most often they are employed to justify if performance is acceptable, (e.g. we found the total error did not exceed 10%). It is far better to use them to determine for instance, that a significant intercept bias of +2 units at the bottom end of the operating range of 1 to 100 units was compensated by a significant slope bias of -6%. The former "laissez-faire" attitude does not promote a healthy attitude towards accuracy control. In order to maintain accuracy one must actively search for inaccuracy. To do so requires a terminology of "error" which recognizes the reality of and the sources of error.

(D. King (416) 248-3015)

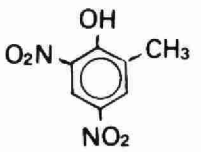
MATERIAL IDENTIFICATION IN THE LABORATORY

1. - Yellow Well Water

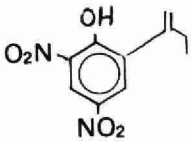
The Organic Trace Contaminants Section was requested to determine the cause of yellow colouration in a rural Ontario drinking well water sample. Preliminary examination of samples submitted to the laboratory indicated that the cause of the colour was contamination by nitro-phenols or hydroxyazobenzene compounds. The colour disappeared on acidification and could be re-developed by the addition of sodium hydroxide. Extraction of the acidified solution with ether gave a yellow extract, which, when spotted on a silica gel thin layer chromatographic plate and developed in benzene-ethyl acetate (2:1), showed only one component to be present.

Evaporation of the ether extract left a trace of yellow oil which was examined by infrared spectroscopy (IR). The IR spectrum clearly indicated the presence of dinitro-phenol. A reference sample of dinitro-orthocresol (DNOC) was found to have an IR spectrum very similar to that of the unknown but it clearly indicated that the unknown possessed a longer aliphatic side chain.

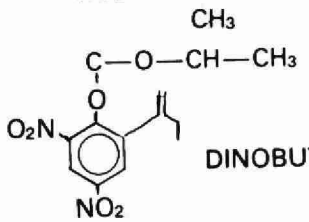
The results suggested that the contaminant might be dinitro-o-sec butyl phenol, DINOSEB, which has applications as an insecticide, ovicide and herbicide. A reference sample of DINOSEB was obtained and it was found that its spectrum was identical with that of the contaminant isolated from the well water. Its identity as DINOSEB was further confirmed by gas chromatography of the methylated contaminant.



DNOC



DINOSE B



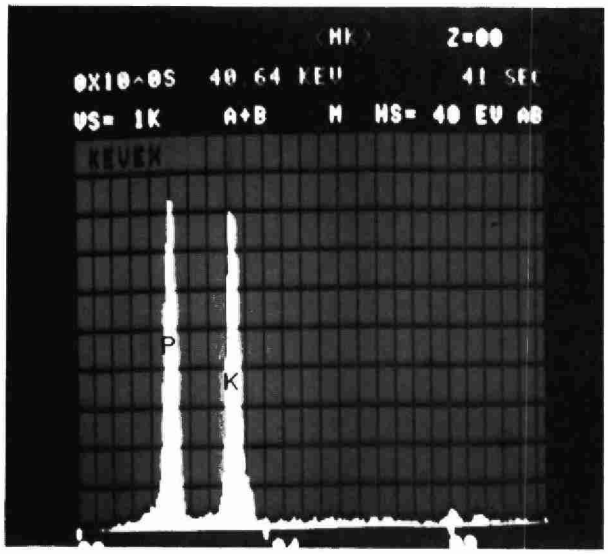
DINOBUTON

Although there were no indications that the identified DINOSEB originated from DINOBUTON (a insecticide), this possibility could not be excluded on the basis of the analytical results.

(G. Wyhovszky (416) 248-3469)

2. Insect Fallout

Owners of property near a shopping plaza complained about a white material being deposited on their cars. A laundry operation located in the plaza was primarily suspected as the emission source. Samples from the automobiles and various soaps, detergents, fabric softeners and so on from the laundry were submitted to the laboratory for analysis. Examination by electron micro probe of the material from the cars showed the particles to contain potassium and phosphorus. None of the laundry materials were found to have a similar composition, thereby excluding the laundry as the emission source. On further inspection of the complaint site, it was observed that the automobiles were parked close to a poplar tree and that the tree had twigs with insect galls. Twig samples were brought to the laboratory and the material in the galls was analyzed. Examination revealed that the insect gall material had the same morphology and chemical composition as that found on the complainants' cars, indicating that the insect infested tree was the source of the deposited material.



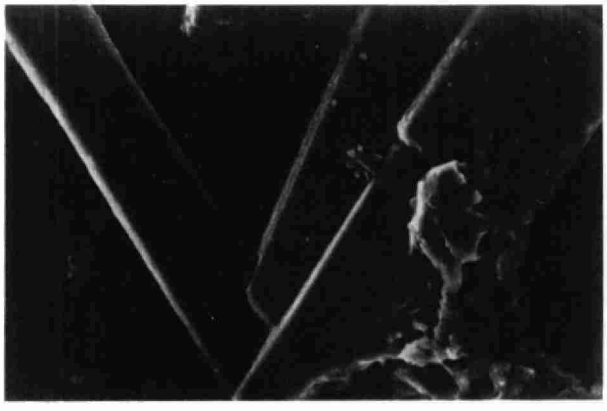
X-ray spectrum showing the presence of K, P, in gall material

SEM photomicrograph of gall material.

3. Crystal Growth

A survey was recently conducted in Nanticoke, Ontario to characterize the air particulate matter and to monitor ambient air quality in this growing industrial area. An Andersen sampler, which simulates the fractionation of air particulate matter in the human respiratory tract, was operated for a period of one week. The samples were collected on clean polyethylene sheets, placed on the various stages of the sampler. These sheets were then submitted to the laboratory for microscopic and chemical examination. The microscopic examination was performed using a polarizing optical microscope and a scanning electron microscope, fitted with an energy dispersive X-ray spectrometer. Chemical analysis was performed by flameless atomic absorption spectrometry. The microscopic examination revealed that the greater portion of the material collected on stages 1 - 5 consisted of particulate matter of non-industrial origin, that is, soil minerals, diatoms, pollen, etc.

In stages 6 and 7, which normally collect particles <1.1 µm in diameter, an interesting phenomenon was observed. These stages were found to contain large crystals which were subsequently identified as ammonium sulphate. Since the crystals, shown in the accompanying electron microscope photograph, were greater than 150 µm in size, there was no way they could have been collected in this form on stages 6 and 7.



There are two possible modes of formation for these crystals: a) sulphuric acid present in the air in fine aerosol form was deposited on these stages and neutralized by gaseous ammonia or b) ammonium sulphate present in a fine aerosol form was deposited on these two stages. These crystals then went into solution during periods of high humidity and later recrystallized when the humidity dropped (e.g. at nighttime). The significance of this finding is being evaluated, in connection with the possibility of sampling of free sulphuric acid in the atmosphere.

(J. Pimenta (416) 248-7101)

4. Fungal Film

A problem of gray-black film formation over several structural units of the Kirkland Lake Water Pollution Control Centre building was brought to the attention of the Microbiology Section. Samples of the black powdery mass were collected from the affected areas, including the painted canvas-jacket water line and roof beams. Microscopic examination revealed that the powdery mass was composed mostly of fungal spores. Upon subsequent culturing on appropriate growth media, samples yielded eight different fungi. Inadequate light, excessive moisture, poor maintenance and availability of sufficient organic material were responsible for the development of fungal contamination. Several short and long term control measures were recommended to overcome this problem.

5. Pink Slime

Water samples (containing pinkish slimy material) from a drinking water fountain in the Parkside Public School in Ajax were submitted to the Ministry's Microbiology Section. A detailed examination (through direct microscopic observations and culturing techniques) of these samples showed the absence of coliforms and iron bacteria. The pink jelly-like substances were composed of fungal hyphae (filaments) and spores, and yielded three different fungi. Extensive cleaning with hypochlorite solutions and maintenance of proper hygienic conditions were suggested as control measures to eliminate this contamination.

(A. Qureschi (416) 248-3008)

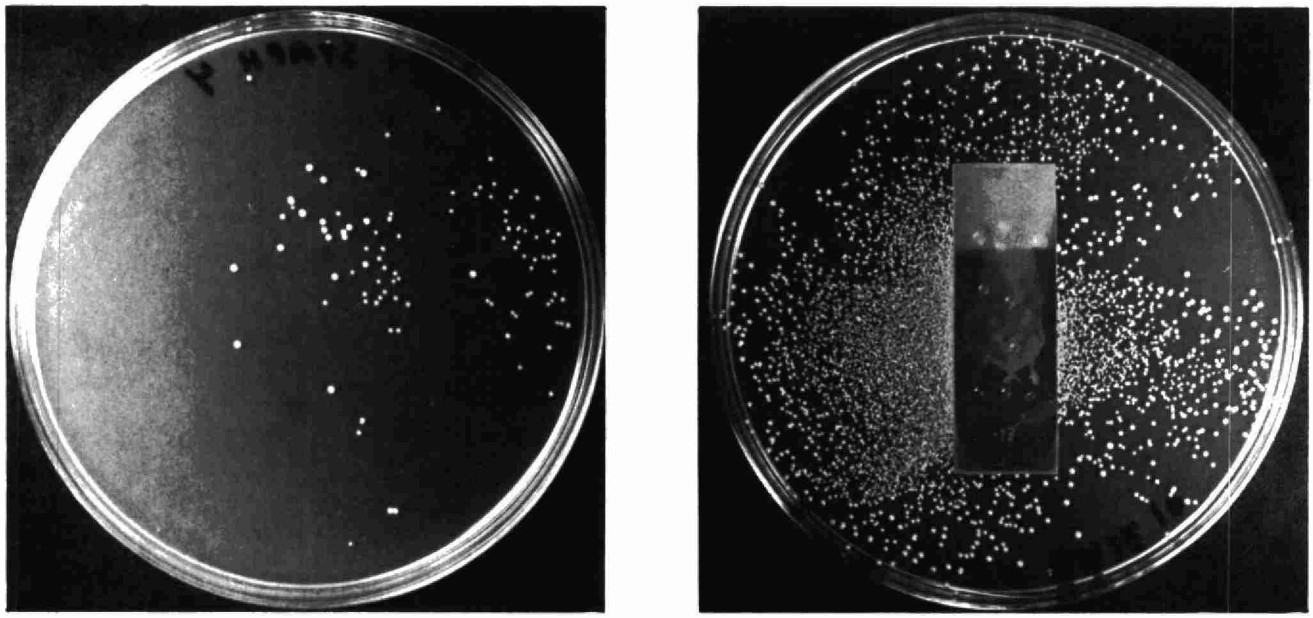
POTENTIAL HAZARDS ASSOCIATED WITH THE BACTERIAL CATALASE TEST

Very little attention has been given in the literature to the potential health hazard inherent in performing certain routine biochemical tests on bacteria. In particular, testing for production of the enzyme catalase depends upon gas libera-

tion, often involving considerable effervescence. This enzyme is present in most cytochrome-containing aerobic and facultative anaerobic bacteria with the main notable exception being Streptococcus spp. Hydrogen peroxide is formed in bacteria as an oxidative end product of the aerobic breakdown of sugars, and if allowed to accumulate, is toxic to bacteria eventually resulting in their death. The method generally recommended for performing the test for catalase production consists in adding a few drops of dilute hydrogen peroxide to a single bacterial colony spread on a glass slide. If immediate bubbling is observed, this indicates that the hydrogen peroxide has been decomposed to oxygen and water via the action of catalase.

The catalase test is normally performed on a glass slide on an open bench and is a routine procedure in the taxonomic identification of bacteria, especially in distinguishing Streptococcus spp. from Staphylococcus spp. The Microbiology Section investigated the possible hazard created by aerosol production.

Using pure culture of Staphylococcus aureus, Escherichia coli and Bacillus sp., replicate tests for catalase production were performed on glass slides placed on the surface of large (18 cm) plates of Trypticase Soy Agar. In this way the presence and extent of aerosol contamination could be demonstrated, as each living cell or clump of cells falling onto the agar would multiply and produce a colony during subsequent incubation at 35°C for 48 hours. Plates of Trypticase Soy Agar were also held, agar side down at heights of 1, 4, 10 and 20 cm above the reaction site for a period of one minute, and incubated under the same conditions.



Growth spread by aerosol liberation around the site of the reaction and at a height of 4 cm above the catalase reaction. (S. aureus).

Growth of each of the bacteria studied was observed around and above the reaction. An example of the extent of aerosol production is shown in the accompanying photographs. Thousands of viable Staphylococcus aureus cells were scattered around the catalase reaction site during the liberation of oxygen. Growth was also observed on plates held as high as 10 cm above the reaction in the case of S.aureus and Bacillus sp. As a result of this study, the recommendation was made that the catalase test be performed on a glass slide contained within a covered petri dish. Without a cover such as a petri dish lid over the slide, the technician would be liable to inhale bacterial cells, which in the case of organisms such as Pseudomonas aeruginosa or Staphylococcus aureus could pose a serious health hazard.

(J.E. Pagel (416) 248-3755)

CHARACTERIZATION AND IDENTIFICATION OF ORGANIC SUBSTANCES IN DRINKING WATER

Organic chemicals in drinking water can arise from naturally occurring organic matter, synthetic chemicals from industrial point sources, synthetic chemicals from nonpoint sources (e.g. runoff from agricultural land, atmospheric deposition) and other contaminants.

Trace organics are of interest because some are known to be detrimental to aquatic life and some are suspected of being detrimental to human health when ingested. Unfortunately, very little is known about the human health effects of chronic exposures to very low levels of organic chemicals in water. Basic to an assessment of human health effects is the best possible documentation of the occurrence of trace organics, as well as accurate measurement of their concentrations in drinking water.

Often in trace organic analysis, only those compounds that are volatile enough to pass through a gas chromatograph are identified. In addition, this group of compounds is further restricted to those that can be extracted from water by a relatively nonpolar solvent such as methylene chloride. Because of these restrictions, only 10 - 20% of the total mass of organics are currently analyzed. However, it would be desirable to identify and quantitate the complete spectrum of organic compounds.

The Ontario Research Foundation has been awarded a two year contract to undertake this work, financed from the Provincial Lottery Trust Fund. The first year of the study will focus on establishing analytical methodology for the concentration, identification and quantification of all trace organics in water.

The second year objectives of the study are to carry out comprehensive surveys of two water treatment plants for organics in the raw and treated water. Variability of the presence and concentration of organics will be studied on a predetermined test basis over the course of a year.

(R.D. Smillie (416) 248-3031)

AN ELECTRON MICROSCOPIC VIEW OF OZONE INJURY TO TOBACCO LEAVES

Ozone was first recognized as a phytotoxic constituent of photochemical smog in the late 1950's. Since then, further investigations have shown that ozone is responsible for injury to many agronomic and horticultural crops, as well as deciduous and coniferous trees. Ozone probably causes more injury to vegetation than any other air pollutant in North America and many susceptible species, such as tobacco, show characteristic foliar symptoms of ozone injury. Such symptoms can sometimes be confused with those caused by pathogenic organisms and can best be distinguished from the latter by careful study of freshly damaged tissue under the light or electron microscope. The electron microscope offers several advantages over the light microscope in such studies because of its greater depth of field, wider magnification range (from 300x to >1,000,000x) and superior resolving power (which is of the order of a few Angstroms).

Ozone is produced in the troposphere by the photolysis of nitrogen dioxide and the concomitant removal of the nitric oxide produced by reaction with hydrocarbon free radicals from incomplete hydrocarbon combustion.

Although it is a normal component of the ionosphere, the increase in automobile traffic over the last three decades has greatly increased concentrations of ozone precursors in the lower troposphere. During the summer months, concentrations of ozone in large, warm air masses can increase from background levels of about 0.01 ppm to hourly average concentrations of 0.08 ppm or greater. Ozone levels in excess of 0.08 ppm, if sustained for several hours, are toxic to several important agronomic crops in southern Ontario, particularly tobacco and white beans. Financial losses to southern Ontario farmers due to ozone sometimes exceed several million dollars per annum.

A tobacco plant showing symptoms of ozone injury was sampled for microscopic investigation. Several tissue samples were taken from an injured leaf for light and transmission electron microscope study. All specimens were fixed in glutaraldehyde-osmium tetroxide fixative, embedded in Spurr's embedding medium and sectioned prior to viewing.

A cross-section through the conjunction of healthy and injured portions of the leaf revealed the presence of a boundary layer (labelled R, Fig. 1). The tissue to the left of the boundary shows collapsed epidermal cells (both upper and lower), and crumpled and distorted palisade and mesophyll cells (cf. healthy cells to the right of the boundary).

At the subcellular or ultra-structural level several modifications in cellular organization were also observed. In the cells of an ozone induced lesion there was a marked thickening of the cell wall from about 0.1 µm (normal thickness) to approximately 1 µm (i.e. x 10 enlargement) accompanied by a disruption and shrinkage of the cytoplasm. What appeared to be spherulites (ordered aggregates of proteinaceous material produced as a response to physiological stress) were also evident in these cells.

Figure 2 shows a portion of a damaged palisade cell. Note the very thick cell wall, (W) the damaged chloroplast with starch grains (S) and the remnants of grana, which are waferlike bodies found within the chloroplasts. There are also large intracellular spaces (I) due to lysis of the protoplast.

The foregoing is a brief description of some of the observed changes in ozone-injured tobacco, and work is continuing to further characterize such phenomena by growing plants in carefully controlled environments and recording the microscopic and ultramicroscopic symptoms.

(A. Hinds (416) 248-7101)

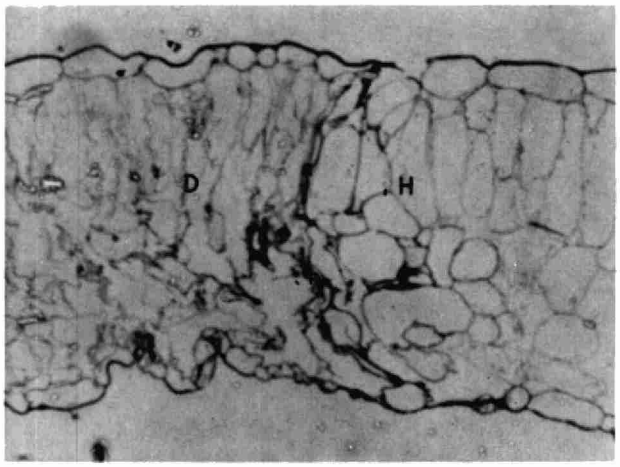


Fig. 1
Light Micrograph (160x) - Conjunction of injured and Healthy tissues.

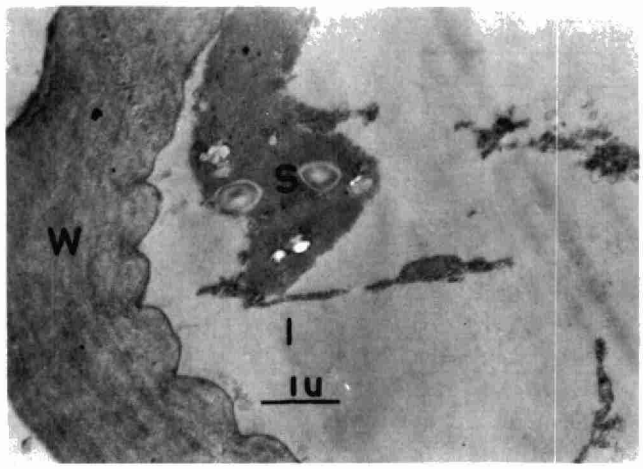


Fig. 2
Electron Micrograph (8000x) Injured cell with thickened cell wall (W) damaged chloroplast containing starch grain (S), and large intracellular space (I).

Recent papers and reports by Laboratory staff overleaf.

RECENT PAPERS AND REPORTS PREPARED BY LABORATORY STAFF

PAPERS, PRESENTATIONS

M. G. McKenney, E. G. Adamek, G. Craig, J. Reinke. An Evaluation of Effluents Generated by a Thermomechanical Pulp Mill. Presented at the 1979 International Mechanical Pulping Conference in Toronto, 1979.

R.D. Smillie and D. T. Wang. A Comparison of Extraction Techniques for Polynuclear Aromatic Hydrocarbon Analysis of Industrial Effluents and Natural Waters. Present at the 4th International Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, Ohio, 1979.

G. A. V. Rees and L. Au. Use of XAD-2 Macroreticular Resin for the Recovery of Ambient Trace Levels of Pesticides and Industrial Organic Pollutants from Water. Bull. Environm. Contam. Toxicol., 22, 561 (1979).

P. Vijan. Determination of Antimony in Environmental Samples by A.A.S. Amer. Laboratory, 11. 32 (1979).

J. E. Pagel and L. T. Vlassoff. Determination of Performance Characteristics for Fecal Coliform Enumeration Procedures. Presented at the 79th Annual Meeting of the American Society for Microbiology in Los Angeles, May, 1979.

B. N. Neary. Overview of Contaminants in Fish. Presented at the Northeastern Region Technical Seminar, Sault Ste. Marie, November, 1979.

B. Loescher. Laboratory Methodology, Collection and Sampling of Precipitation Samples. Presented to the Federal/Provincial Task Force on Acid Precipitation, Toronto, June, 1979.

A.B. Foster. The Selection and Application of Filter Media to Air Sampling. Chemical Institute of Canada Symposium on Air Sampling in Ottawa, January, 1979.

O. Meresz. Environmental Organic Chemistry. Presented at Trent University, Peterborough, September, 1979.

A. B. Foster. Inorganic Trace Contaminant Analysis for Air Contaminants. Presented at the Sampling and Monitoring Techniques for Source and Ambient Air Contaminants, Toronto, April, 1979.

O. Meresz. Organic Trace Analysis for Airborne Contaminants. Presented at the Sampling and Monitoring Techniques for Source and Ambient Air Contaminants, Toronto, April, 1979.

O. Meresz. Environmental Organic Chemistry and Trace Analysis. Presented at York University, Toronto, October, 1979.

M. Young, B. Neary and P. L. Diosady. Studies on the Microbial Methylation of Mercury in Sediments from the St. Clair River and Lake. Presented at the 29th Annual Meeting of the Canadian Society of Microbiology in Victoria, B.C., June 1979.

J. A. Clark. The Relationships of 35°C Membrane Filter (MF) Plate Counts and m-Endo Agar LES MF "Background Counts" to the Detection of Indicator Organisms by the MF and Presence-Absence (P-A) Procedures. Presented at the 29th Annual Meeting of the Canadian Society of Microbiology in Victoria, B.C., June, 1979.

G. S. Hendry and S. Janhurst. Discharge of Pseudomanas aeruginosa and Klebsiella pneumoniae to the Sturgeon River from a Forest Products Mill. Presented at the 79th Annual Meeting of the American Society for Microbiology in Los Angeles, May, 1979.

A. A. Qureschi and B. J. Dutka. Stormwater Runoff Microbiology Adds to Concerns. Water and Sewage Works, 126, 86 (1979).

A. A. Qureschi and B. J. Dutka. Microbiological Studies on the Quality of Urban Stormwater and Runoff in Southern Ontario, Canada. Water Res., 13, 977 (1979).

J. Crowther, F. D. Tomassini and J. McBride. Application of Ion Chromatography to the Analysis of Environmental Samples. Presented at the Canadian Laboratory Management, Planning and Design Conference in Toronto, November, 1979.

REPORTS

R. F. Bonner, O. Meresz, B. Shushan and T. Sakuma. The Aqueous Chlorination of Biphenyl-Possibilities for PCB Production in Sewage Treatment Plants.

R. D. Smillie and D. T. Wang. Determination of Polynuclear Aromatic Hydrocarbons in Fish. September, 1979.

M. G. McKenney, E. G. Adamek, G. Craig and J. Reinke. An Evaluation of Effluents Generated from a Thermomechanical Pulp Mill. November, 1979.

G. A. V. Rees, G. Crawford and R. Brunato. An Inventory of Data on PCB and Mirex Levels in Ontario Fish. Addendum 1-79. August, 1979.

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S. Janhurst, G. Myslik and A. A. Qureschi. The Water Quality of Eagle (Machar) Lake, South River, Parry Sound District. 1979.

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